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TISSUE CULTURE OF OPIUM POPPY HYPOCOTYLS

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Abstract

Hypocotyls of *Papaver somniferum* exhibited growth only when inoculated on ½ x MS medium. Callus induction occured on the addition of auxin and a kinetin. Best callus resulted at 0.5 mg/L of 2,4-D and 1.0 mg/L of K in presence of coconut milk. Early browning and death of tissue was controlled by the addition of sodium diethyl dithiocarbamate at 250 mg/L. However, the addition of this substance necessitated a change in the hormone concentration and an excellent callus resulted at 0.1 mg/L of 2.4-D, kinetin 2.0, SDD 250 mg/L and CM at 5% (v/v). The cultures remained alive for a longer duration on this combination and could be easily subcultured.

Introduction

Papaver somniferum L., is an annual plant belonging to the family Papaveraceae and is being cultivated on a commercial scale in countries like Burma, India, Iran, Pakistan, Turkey, Yugoslavia, Japan etc. Opium poppy is economically important for Pakistan in general and the North-West Frontier region in particular, where it occupies about 3-4% of the total cultivated area. The World Health Organization is seriously considering replacing *P. somniferum* with *P. bracteatum* (Shiio & Ohta, 1973). It is known that *P. somniferum* has the metabolic capacity to produce morphine and codeine, while *P. bracteatum* lacks this property and thus the end alkaloidal product is thebaine.

Tissue culture of different explants of poppy have been established eg. placenta (Pontovich, 1959), seedling root, stalk and capsule (Furuya *et al.*, 1972). Moreover, presence of alkaloids has been detected in callus tissues (Ranganathan *et al.*, 1963; Carew & Staba, 1965; Staba, 1969). Some of the alkaloids metabolied by these tissues have been isolated and identified (Furuya *et al.*, 1972).

The present studies comprise part of a project of the somatic hybridization of *P. somniferum* and *P. bracteatum in-vitro*. This necessiated cultural studies of both the species. In the present communication callus formation on opium poppy hypocotyls is reported.

Materials and Methods

a) *Plant Material*: Seeds of a high yielding opium poppy (*Papaver somniferum* L.) were obtained from the Narcotics Board, Bunir.

b) *Cultural Studies:* The seeds after washing in tap and distilled water to remove dust and other macrocontaminants, and surface sterilization with alcohol and mercuric chloride were inoculated on plain agar containing 2% sucrose in culture tubes (6x1"). The seeds germinated after 5 days and explants thus obtained were transferred to test media when the cotyledons had fully expanded.

c) *Culture Media:* The following media viz., White (1934) and Murashinge & Shoog as modified by Gamborg & Wetter (1975) were used. The media contained the following addenda wherever mentioned: Coconut milk (CM), Yeast extract (YE) and various antioxidants. Different growth hormones were supplied through the nutritional medium in concentrations mentioned in the text in addition to 0.8% Difco-Bacto agar containing 2% sucrose and the final pH was adjusted to 5.8. Erlenmeyer flask and culture tubes respectively, containing 50ml & 25 ml of the medium were inoculated with one explant in each. The cultures were grown in an illuminated chamber having 18 hours light in 24 hours cycle at 25 ± 1°c.

Results

The seedling after 8 days of germination developed a root system, a hypocotyl, of 2cm long, and fully expanded cotyledons. Those plants which had produced leaves were discarded. The root and the cotyledons alongwith the apical meristem were removed before inoculation. Thus the explant consisted of about 1 cm of the hypocotyl.

The explant did not exhibit any growth when inoculated on either Whites or MS medium without any addenda and died after about seven days. Addition of various addenda to the above media was not good, when the salt concentration was at full strength. Whites medium at ½ x exhibited a poor growth of the explant compared to the ½ MS medium. MS at half strength, was therefore, used as the basal medium

Two auxins, viz., Indoleacetic acid (IAA) and 2, 4-Dichlorophenoxyacetic acid (2, 4-D) were used at 0.1, 0.5 and 1.0 mg/L, while two cytokinins: Benzylaminopurine (BAP) and 6-furfuryl-aminopurine (K) were used at 0.1, 0.5 1.0 and 2.0 mg/L. Either of the growth hormone were unable to support a reasonable growth of the explant when supplied alone. Auxins at 1.0 and Cytokinins at 2.0 mg/L proved to be toxic. Of the auxins, IAA could not induce any growth, rather the explant died after 1 week. Similarly BAP was also without any effect on callus induction either alone or in combination with auxins. Kinetin and 2, 4-D, were without any appreciable effect on callus induction at lower concentrations. Though some growth took place at higher concentrations (i.e 0.5 mg/LK and 1.0 mg/L 2,4-D), the tissue turned brown after four weeks of culture and deteriorated further with prolongation of the incubation period. Even subculture of the tissue on fresh medium could not check this deterioration and it died after 8 weeks in both the cases.

Table 1. Callus induction and its further growth on ½ MS Medium containing 5% v/v CM and the hormones in various concentrations.

Growth hormone mg/l 2,4-D	K	Callus formation	Remarks
0.1	0.1	–	Tissue died after 3 weeks
0.1	0.5	+	Callus turned brown & died after 10 weeks
0.1	1.0	+ +	Callus alive but turning brown
0.5	0.1	+ +	Same as above
0.5	0.5	+ + +	Callus turning brown after 6 weeks
0.5	2.0	+ +	Callus deteriorates after 8 weeks
1.0	0.1	–	Callus growth stopped, tissue died within 3 weeks
1.0	1.0	+ +	Callus commenced browning after 10 weeks. Further growth stopped.
1.0	2.0	+ +	Same as above.
1.0	4.0	+ +	No appreciable change from the above condition

–, No callus formed
+, Slight callus formation
++, Little callus formed
+++, Medium callus formed
++++, Good callus formed
+++++, Excellent callus formed

CM, Coconut milk

Addition of coconut milk (CM) at 5% was found to be useful on callus induction and its further proliferation. The medium supplemented with low concentrations of growth hormones did not support good growth (Table. 1), but a gradual increase in concentration exhibited effects. Kinetin at 1.0 mg/L and 2, 4-D at 0.5 mg/L in presence of CM was found to be the best for callus induction on explants. However, it was not suitable for further proliferation of the callus. Decreasing 2,4-D level to 0.1 ml/L proved to be the best for callus growth and this medium supported formation of white, friable callus during sucessive subcultures. The concentration of K at 1.0 and 2,4-D at 0.5 mg/L which induced callus formation on explants, was inferior in callus growth to the medium containing K (1.0 mg/L) and 2,4-D (0.1 mg/L). Further increase in auxin concentration lead to early browning and decreased growth rate of the callus.

To avoid early browning of the tissue, various antioxidants were added to the medium viz., i) mixture of amino acids; Cystein-HCl (2.5) tyrosine (2.5) and glycine (10 mg/L); Polyvinyl pyrrolidine at 250 mg/L or) Sodium diethyl dithiocarbamate (SDD) at 250 mg/L. Of the antioxidants used, SDD proved to be the best, as it supported a friable, white callus for more than 3 months. However, a variation in the concentration of growth hormones did alter this effect. Furthermore, proliferation and callus growth exhibited a change over that medium containing CM only. Kinetin (2.0), 2,4-D (0.1 mg/L), CM (5%, v/v) and SDD (250 mg/L) supported excellent callus growth (Table 2). Kinetin when used at 1.0 mg/L alongwith various cncentrations of 2,4-D could check browning of the callus but an increase in auxin level exhibited an adverse effect as a decrease in callus growth was noticed. An increase of K to 2.0 mg/L enhanced callus proliferation and early browning of the tissue was noticed. Cultures raised in SDD medium could be easily subcultured even after 15 weeks without exhibiting any signs of death or decrease in potency.

Table 2. Callus induction and its further growth on ½ MS Medium containing 5% v/v CM + sodium diethyl dithiocarbamate (SDD) (250 gm/l) and the hormones in various concentration

Growth hormone. mg/l		Callus formations	Remarks
2,4-D	K		
0.1	1.0	+ + + +	Whitish friable callus turning brown after 4 months
0.1	2.0	+ + + + +	-do-
0.5	1.0	+ + + +	-do-
0.5	2.0	+ + +	-do-
1.0	1.0	+ + +	-do-
1.0	2.0	+ + +	-do-

Discussion

The results indicate that neither Whites nor MS medium were conducive for callus formation. It needed addition of some growth factors, especially a combination of an auxin and kinetin and some other addenda. Moreover. the salt concentration had to be reduced for a healthy growth of the cultures. 2,4-D was without any effect at the concentrations when used either alone or in combination with the cytokinin. These results are in contrast to those reported for *Rauwolfia serpentina* (Ilahi & Akram, unpublished). Similarly cultures of tobacco pith (Das *et al.,* 1956), Gerbera (Murashige *et al,* 1974) and tobacco explants (Murashige & Skoog, 1962) exhibited marked cell prolifertation in the presence of suitable concentrations of auxin and cytokinin. However, callus growth enhanced when CM was supplied at 5% v/v. whereas a concentration 10% of CM proved toxic because it inhibited callus growth.

Tissue browning, probably due to the production of polyphenols, was a wide-spread phenomenon in poppy cultures. The tissue showed inhibition of growth even on transfer to fresh medium. Use of sodium diethyl dithiocarbamate proved to check the phenomenon of early browning as reported for other plant species (Krishnamurti, 1981; Rao & Bapat, 1980; Amin, 1982; Ullah, 1982).

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